OBSERVATIONS UPON THE OCCURRENCE, STRUCTURE, AND FUNCTION OF THE GIANT CELLS OF THE MARROW.

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THE observations which this communication is intended to record were made in the course of a study of the hæmatopoietic function of the marrow, the results of which have been given in the previous paper. The giant cells have been supposed by some, notably by Foa and Salvioli (1), to have a direct connection with the production of new red corpuscles; so that an investigation of this last subject necessitated a more or less thorough consideration of the giant cells. My observation convinced me that no direct connection exists between the giant cells and the newly formed red corpuscles. The reasons upon which this conclusion is founded, together with certain interesting facts not heretofore observed relating to these peculiar cells, forms the excuse for the present brief paper, which for the rest does not attempt to give any final solution to the problem of their function in the marrow. My observations have been confined to the cat, as this was the animal which was found to be most convenient for the study of the blood corpuscles. The giant cells, moreover, seem to show certain differences in structure in different mammals (Werner), so that by restricting the work to one animal the different preparations could be more easily compared amongst themselves.

Before attempting to give a description of these cells as found in the cat's marrow, it is necessary to insist upon a division of them into two classes, — a distinction which has been emphasized before by certain writers, but which is forgotten or denied by others. We have, in the first place, giant cells containing a variable number of separate nuclei. These form what are generally described as giant cells or myeloplaques, and have been found not only in the marrow, but also in a number of pathological formations, tubercle, syphilis, etc. In the second class, the cell contains not many, but one huge nucleus, often bent or coiled upon itself, or imperfectly segmented or notched so as to form a complicated structure.

These have been described as giant cells with budding nuclei (Bizzozero [2]); but it seems to me that they are worthy of a more distinctive name. I shall speak of them hereafter as megakaryocytes, or large nucleated giant cells, while the first class might be named polykaryocytes, or multinucleated giant cells. Some of the German histologists, especially Arnold (3), have held that transitional forms can be found between the two classes, and look upon them therefore as two stages in the life-history of a single cell. The first stage is supposed to be the large nucleated form, and this by fragmentation passes into the multinucleated form. My observations upon the cat have led me to believe that we have here two entirely different cells, probably with different functions, and that what are described as transitional forms, which I have also seen, though very rarely, are such only in appearance, and can be accounted for more easily upon other grounds, possibly as cells undergoing degenerative changes.

In the cat the polykaryocytes are found in great numbers in the developing bone of the fœtus, and are usually seen in sections lying upon the spicules of bone while in process of formation. In extra-uterine life they can be found also, but only in the marrow of the spongy bone, in the neighborhood of the bony dissepiments. I have never met with them, in the cat, lying in the mass of red marrow which fills up the interstices of the spongy bone and forms solid plugs at the ends of the medullary cavity of the long bones. It is well known, also (4), that when pieces of sponge or other porous substances are introduced into serous cavities, the leucocytes swarm into the interstices of the foreign body, and in a short while multinucleated giant cells are found lying upon the partitions, just as in the bone they lie upon the bony spicules.

Very many hypotheses have been made as to the origin of these cells, the chief question being whether they are derived from the growth of a single small cell or from the fusion of a number of separate cells. It is not necessary for me to give here any detailed account of the views that are held upon this question, as I have no special observations of my own to offer which might help to solve the problem. My own opinion has been that this form of giant cell is produced by the fusion or amalgamation of a number of smaller cells. I have been led to this view chiefly from the fact, already mentioned, that they seem to be found only when lying upon some solid substratum, such as the septa of sponge or the spicules of spongy bone. In the latter locality, when the spicules of forming bone are covered with an epithelium of osteoblastic cells, it seems plausible to think that these closely packed cells might become forced to form a polykaryocyte, and a number of apparently transitional steps in this process can be seen in sections of the femur in fœtal cats about 9 cms. long. The rows of osteoblastic cells are found in the same localities with the polykaryocytes, and the nuclei of the cells have the same vesicular character in both kinds of cells. As far, then, as my observations upon this cell have gone, they have induced me to side with those who believe they are derived from the fusion of small cells. The function of these cells is unknown. The common view that they are concerned in the absorption of bone (osteoclasts) seems to me to rest upon very slight evidence. If we find them in developing bone lying upon the cartilage trabeculæ which are being absorbed, we find them also on the partitions of sponge or pith, introduced into serous cavities where no absorption is taking place; and the conclusion in the first case that the absorption which is going on is due to the giant cells (osteoclasts) is illogical. Absorption of tissues is an occurrence common enough in the body, and it is difficult to understand why the absorption of bone or cartilage should require the activity of a special cell, when the absorption of other tissues does not. It would seem more probable that this form of cell has no specific function, and that its formation is, in fact, accidental or, in a certain sense, pathological: that the presence of a solid substratum leads to an abnormally rapid growth of lymphoid cells, leucocytes, osteoblasts, as the case may be, and the fusion of some of these to produce multinucleated giant cells. The same explanation might hold, as far as I can see, to the occurrence of this form of cell in pathological formations, except that in these cases the too rapid growth is brought about by other conditions.

The large nucleated giant cell, or megakaryocyte, unlike the multinucleated form, is found, and found abundantly, in the midst of the red marrow filling up the ends of the long bones and the spaces between the trabeculæ of the spongy bone. It does not lie upon the spicules of bone or cartilage, but away from them, surrounded by marrow cells, nucleated red corpuscles, blood corpuscles, and the other cells characteristic of marrow. It appears in the marrow with its first formation, as shown by cross and longitudinal sections of the femur in an embryo cat 9 cms. long, and is then surrounded by the elements of the marrow. Throughout the rest of the animal's life it can be discovered in sections or teased specimens of the red marrow. It is evidently a peculiar kind of cell, which has some definite function to perform. Not only is it found in the marrow, but throughout embryonic life it is met with in abundance in the liver and the spleen as long as these organs have any distinct connection with the production of red blood corpuscles; and it occurs more abundantly, the more active the blood-forming function of the organ. In histological structure it is the same in the embryonic liver and spleen as in the adult marrow. This fact has been stated by others (Foa and Salvioli [1], etc.), and is perfectly evident to any one who will take the trouble to look. It is certain, therefore, that the function of this cell is the same in the embryo spleen or liver as in the adult marrow. That it is not simply one stage in the life of the multinucleated giant cell seems to be demonstrated by the fact that typical multinucleated cells are never found in the mass of red marrow, in the cat at least, lying in the cavity of the long bones, nor in the embryonic liver or spleen, though the megakaryocytes are so numerous.

The structure of the megakaryocytes has been clearly described by a number of observers, especially by Arnold (3). They are giant cells, each with a huge nucleus. The body of the cell is finely granular, and shows no special peculiarities in structure. It is interesting to note that it does not possess the power of amœboid contraction which is so marked in many of the marrow cells, especially those with elongated nuclei. The nucleus is the characteristic part of the cell, but varies considerably in size, complexity, and minute structure (Fig. 11, *a, b, c, d*). Frequently it is crescent-shaped, or even makes a ring;

at other times it is coiled upon itself, or appears simply as a large, central mass, with projections from its surface; but in most cases it shows incomplete constrictions of or partitions from the peripheral membrane or layer of chromatin, which tend to separate it into small nuclei comparable to those of the typical marrow cells. The nucleus is granular, and in well-preserved specimens shows a distinct chromatin reticulum, with conspicuous nodal points. In addition, one or many nucleolar masses may be present, sometimes one apparently for each incomplete small nucleus into which the large mass is divided, sometimes only one for the entire nucleus, or in some cases none at all. What I have called nucleolar masses or nucleoli are distinguished from the large granules or nodal points of the chromatin reticulum by their staining. In the triple stain of hæmatoxylin, eosin, and saffranin, the chromatin reticulum takes the hæmatoxylin stain as in nuclei generally, while the nucleoli, like the nucleoli of the marrow cells, show a preference for the saffranin, staining bright red when the time of exposure to the different stains is properly adjusted. In a number of instances, in the sections of the normal marrow of adult cats, megakaryocytes were met with in which the nuclei showed no chromatin reticulum at all, but stained diffusely or almost so with the different dyes employed, taking the stain like the nuclei of the matured form of nucleated red corpuscle described in my paper upon the development of the red corpuscles. In such cells where the chromatin was diffusely scattered throughout the nucleus it frequently happened that the nucleus was fragmented (Fig. 1, *e*, *f*). It seems to me that this appearance of the nucleus here as in the nucleated red corpuscles is a sign of old age and death, and that these cells are in process of dissolution, hence the fragmentation of the nucleus. Arnold takes the directly opposite view, and considers this appearance as one of the initial changes leading to a fragmentation of the nucleus and division of the cell into smaller marrow cells.

According to Arnold, the following successive changes in the structure of the giant cell occur, leading up to its fragmentation. 1. The first stage is characterized by an increase in the chromatin substance, the chromatin filaments become more numerous, form networks, etc., and toward the end of the stage a division of the chromatin occurs, leading to a more or less diffused coloration of the nucleus. 2. The periphery of the nucleus becomes indented, and in many cases there is such an important increase in the diffused chromatin substance that the filaments can no longer be distinguished. The indentations of the periphery of the nucleus occur at many points and advance toward the middle, forming the complicated nuclear figures so characteristic of these cells. 3. The chromatin substance becomes concentrated at different points, forming small, dark-colored nuclear bodies which are united by colorless bands. By a continuation of these changes a number of entirely independent nuclei are formed. 4. The protoplasm segments round the newly formed nuclei, either endogenously or by constriction from the periphery. This makes up the process designated by Arnold as "indirect fragmentation," and it is, according to him, the normal method of development or multiplication of the giant cells. He admits in addition, and quotes from Martin and Waldstein to support the statement, that the multinuclear giant cells may reproduce also by true mitotic division of the nucleus, but thinks that this method of division is very rare. I have never myself seen any indication whatever that the nucleus of the giant cells divides by karyokinesis, though I have examined many sections of marrow from cats of all ages, normal, bled, and starved, so that with this animal at least it must be an exceedingly rare occurrence. Moreover, my observations upon the giant cells have never given me any evidence of the correctness of Arnold's view that these cells normally undergo indirect fragmentation. Foa and Salvioli also thought that the megakaryocytes break up by segmentation to form a number of colorless or hyaline cells, which in turn develop into nucleated red corpuscles, and I shall speak further of their theory in discussing the function of this form of giant cell.

On the other hand, that the megakaryocytes multiply by division, like other cells, giving rise to two daughter giant cells, has been clearly proved by my sections. In quite a number of cases the sections have shown me megakaryocytes with two large nuclei at the ends of the cells, and a constriction beginning between them, or, more frequently, two megakaryocytes lying side by side with the line of demarcation between them complete, but the cells still adherent to each other (Figs. 2 and 3). Similar appearances have been seen and figured by Werner (5). In none of the cases of division observed by me was there any indication of karyokinetic figures; hence it is fair to conclude that this form of cell multiplies by direct division. The clear proof furnished by these observations that it does increase by division is also another indication that the megakaryocyte is a definite cell form, and not one stage in the formation of the multinucleated giant cell.

What now is the origin and function of this cell? Believing that the megakaryocyte has no genetic connection with the polykaryocyte, the theories of the origin of the latter have no bearing upon the former. My sections, especially those made through the femur of a fœtal cat, 9 cms., at a time when the marrow was just beginning to form, gave me a number of apparently transitional forms between typical megakaryocytes and the small marrow or embryonic cells. A series of drawings, showing apparently the gradual development of the small cell into the giant cell, is given in the Fig. 4. The drawings were made from different portions of the section, and the theory they suggest is that the small cell enlarges, the increase affecting both the nucleus and the cell substance, and, after reaching a certain size, indentations of the periphery of the nucleus appear, or in many cases ingrowths of the peripheral chromatin, which give the incompletely segmented appearance to the nucleus that is so characteristic, and which has given to them the name of giant cells with budding nuclei. The megakaryocyte is not formed, then, by the fusion of a number of smaller lymphoid cells, nor from a single cell which increases in size by engulfing other similar cells. Both these theories might apply to the polykaryocytes, but certainly not to the megakaryocytes. These latter are developed by the steady growth in size of a smaller lymphoid cell, and the curious structure of the nucleus follows after it has reached a certain size in consequence of partial constrictions or divisions, which are never carried so far, however, as to lead to a complete separation.

With reference to the function of these cells, several different views have been proposed. Arnold and others, who believe that the megakaryocyte becomes ultimately a multinucleated giant cell, believe that the latter constricts off or separates into smaller marrow cells. From this standpoint, the function of the large nucleated giant cell is simply that it forms one stage in a peculiar method of development of the lymphoid cells of the marrow. Löwit (6) speaks of the giant cell — including both varieties — as having some connection with the degenerative changes of the leucocytes, though as far as I know the exact nature of the relationship is not described. He gives three reasons for this view. 1. They are less frequent in the embryo than in the marrow of the adult, and the younger the embryo, the fewer the number found in the liver and spleen. The first part of the statement I cannot corroborate, as in the embryo liver, especially when at the maximum of its hæmatopoietic activity, the giant cells (megakaryocytes) are quite as numerous as in the adult marrow. The second portion of the statement is true to a certain extent. The number of giant cells (megakaryocytes) varies directly with the blood-forming activity of the organ, so that they are not numerous in the liver at its first formation nor toward the end of fœtal life. 2. He has never been able to find them in the lymph glands, even after severe bleeding. In this, Löwit is confirmed by a number of other observers who have stated their inability to find giant cells in the lymph glands. Indeed, this assertion may be accepted as satisfactorily demonstrated, but its bearing upon Löwit's theory of a connection of the giant cells with the degeneration of the leucocytes seems to be very remote. While it is true that we do not find in the lymph glands either giant cells or degenerating leucocytes, that does not in any way prove that the giant cells have any relation to the degenerative changes of the leucocytes. 3. In sections of the marrow, the giant cells are not found where erythroblasts and leucoblasts are in active formation, but rather where the latter show signs of degenerative changes. This statement I cannot confirm; indeed, it seems to me that the reverse is true, as far, at least, as the erythroblasts are concerned, while with the leucoblasts I have not been able to notice that in the neighborhood of the giant cells there is any increase in the number of them undergoing degeneration. Foa and Salvioli have thought that they were able to demonstrate a connection between the giant cells and the nucleated red corpuscles. Their view, as has been stated, is that the giant cell breaks up into a number of hyaline cells, — erythroblasts, to use Löwit's terminology, — which in turn develop into nucleated red corpuscles. Their strongest evidence for this view is the fact that whenever, in the fœtus or in the animal after birth, there is an undoubted formation of nucleated red corpuscles, there the giant cells — megakaryocytes — are also found. This connection has been noticed by a number of observers, and is certainly very constant and striking. In the embryo liver, the embryo spleen, the adult marrow, and in the spleen of the adult during regeneration after partial excision (7) we find megakaryocytes and nucleated red corpuscles side by side. Nevertheless, I have never been able by the most careful and thorough observation to find any actual connection between these two histological elements, or between the giant cell and the erythroblast. Foa and Salvioli picture a group of nucleated red corpuscles supposed to be derived from the breaking up of a megakaryocyte, but groups of the kind figured are in reality derived from the multiplication of nucleated red corpuscles by division. Arnold has stated that white corpuscles are constricted off from the giant cells, but supposes that these corpuscles are not progenitors of the nucleated red corpuscles. In my own sections and teased preparations I have never been able to find any indication that the nucleated red corpuscles are budded off from the megakaryocytes, and no satisfactory example of the derivation of a lymphoid corpuscle of any description from them. In the marrow of the adult, after repeated hemorrhages, where the production of red corpuscles has been vastly accelerated, one would surely expect to see some undoubted sign of the derivation of the nucleated red corpuscle or its colorless predecessor from the giant cell, if it is the function of this last cell to serve as the origin of the new red corpuscles that are being formed. My failure to find any perfectly clear examples of such a derivation has compelled me to believe that the megakaryocytes take no direct part in the production of new red corpuscles.

In a few cases I have obtained giant cells evidently belonging to the class of megakaryocytes in which a smaller portion of the nucleus seemed to be completely separated from the main mass and was lying free in the cell, but always in such cases there was some possibility that the appearance was deceptive. I was not able to obtain a sufficient number of clear cases to convince me that this is a normal occurrence in the life of these cells, though at the time I was convinced that their most probable function was to form the erythroblasts or progenitors of the nucleated red corpuscles; indeed, I abandoned the theory reluctantly because the evidence seemed to be opposed to it, or at least did not support it. If we adopt the compromise view that the giant cells furnish some of the erythroblasts while others, and probably most of the others, arise in a different way, then we could understand why in rapid regeneration of the blood after bleeding it is so rare to find giant cells in the act of producing erythroblasts. But it does not seem probable, to me at least, that these cells should be produced in one organ by two different methods. Nevertheless, the constant presence of megakaryocytes in the blood-forming organs induces me to believe that they have some function to perform in connection with the formation of blood. This is rendered more probable by the fact that in the embryo, at least, the megakaryocytes can be found in the newly forming blood-vessels surrounded by developing blood corpuscles. I have found this in sections of the liver of an embryo cat where, as has been described in another paper, the nucleated red corpuscles and the erythroblasts lie in cords which are destined to become the future blood-vessels of the liver. In some cases, in fact, the cords may be seen to end in channels filled with coagulated plasma and red corpuscles, with or without nuclei. Now, in these cords of blood cells I have found the megakaryocytes, showing that they are connected in some way with the blood (Fig. 5). In longitudinal sections of the hind leg of the same embryo I have found developing blood-vessels lying among the embryonic muscle fibres and in the blood-vessel giant cells, — megakaryocyte, — as shown in Fig. 6. It is very hard to understand what this cell is doing in such a place if it is not connected with the production of either the formed elements or of some of the chemical constituents of the blood.

As I have just said, I cannot find any corroborative evidence for the first view, and am therefore inclined to look favorably upon the second; namely, that the function of the megakaryocyte is to manipulate, in some way, the material of the plasma or lymph, forming some substance for the nourishment of the developing blood cells. This view was suggested to me by a curious phenomenon which I have occasionally found in connection with these giant cells, and which, so far as I know, has not been noticed before. In many sections of the marrow, but especially in sections through the bone and marrow of the femur of a fœtal cat (9 cm. long), I have seen the megakaryocytes, either singly or in groups, with a delicate reticulum radiating out from them on all sides, and enclosing within its meshes the other elements of the marrow. A sketch of this appearance is given in Fig. 7. I should add that Werner has described but not figured what appears to be the same reticulum. In the young developing marrow this appearance is so common and so striking that I thought at first the megakaryocytes had for their function the formation of a supporting reticulum for the marrow, secreting it, as it were, from the cell substance. But when examinations were made of teased specimens of the fresh marrow of young kittens to find if possible whether a giant cell with its reticulum could be teased out from the other elements, I obtained the cells, surrounded not by a reticulum, but by a very large envelope of exceedingly fine and pale material (Fig. 8). Round the nucleus of the cell was the ordinary granular protoplasm forming the body of the cell, but outside of and surrounding this was a large envelope of much more delicate and hyaline material, which did not stain with methyl green. As this was watched under the microscope, in a very dilute NaCl solution of methyl green, vacuoles began to form in it (Fig. 9), and becoming rapidly larger, finally made a reticulum such as I had found in my sections surrounding the cell. This convinced me that the reticulum seen in the sections arose from the action of the fixing and hardening reagents upon this secretion from the cell. The theory that the giant cells make a reticulum is rendered improbable, also, from the fact that they occur in the developing blood-vessels of the embryo. In many cases in the teased specimens the action of the reagent had gone so far that the giant cells were found surrounded only by vesicular-like bodies arising from the vacuolation of the secreted material, as shown in Fig. 10. It seems to me that this broad envelope of material surrounding the megakaryocyte, and evidently formed by it, is very significant. As I found it, no nuclei were scattered through it, and hence the most natural explanation is that it is a material secreted by the cell which is finally dissolved in the plasma, and is used, possibly, for the nutriment of the blood-forming cells; though this, of course, is mere speculation. In sections nothing remains of the material except the reticulum, and this does not stain with any of the reagents used, — alum carmine, hæmatoxylin, eosin, saffranin, Ehrlich-Biondi's stain, indigo carmine (Shakspeare-Norris stain), — or at least stains much more feebly than the protoplasmic cell substance.

SUMMARY.

The contents of the paper may be summed up briefly as follows:

1. Giant cells fall into two classes: *a.* Polykaryocytes, or multinucleated giant cells found in developing bone, in pathological formations, or porous bodies kept in lymph cavities, etc.; *b.* Megakaryocytes, or large nucleated giant cells found in the red marrow of the adult and in the blood-forming organs, liver, spleen, etc., of the embryo.

2. The polykaryocytes have no special function, are not related to the megakaryocytes, and are formed by the fusion of smaller cells in consequence of too rapid growth.

3. The megakaryocytes form a peculiar class of cells. They arise from the growth of small lymphoid cells, and afterwards reproduce by direct division. During their life they form a secreted material which can be seen for a time by the microscope, but finally dissolves in the plasma.

They seem to take no direct part in the production of nucleated red corpuscles or erythroblasts. After a certain period the nucleus alters in such a way that it stains diffusely and then fragments. This seems to be a degenerative change, and probably ends in the total disintegration and dissolution of the cell.

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EXPLANATION OF PLATE.

FIG. 1. *a, b, c, d.* Drawings of megakaryocytes, to show some of the variations in structure of the nucleus. *e, f.* Megakaryocytes in which the nucleus stains diffusely and fragments into smaller pieces, explained as degenerative changes.

FIG. 2. Three megakaryocytes; two in the act of dividing; from a camera lucida sketch.

FIG. 3. Two megakaryocytes; division complete, but the cells still connected; from a camera lucida sketch.

FIG. 4. Four cells from the developing marrow in a section of the femur of a fœtal cat 9 cms. long, stained with saffranin, and intended to illustrate the development of a megakaryocyte from a marrow corpuscle.

FIG. 5. Section of the liver of a cat embryo 2.7 cms. long, showing a megakaryocyte lying in a developing blood vessel, and surrounded by erythroblasts.

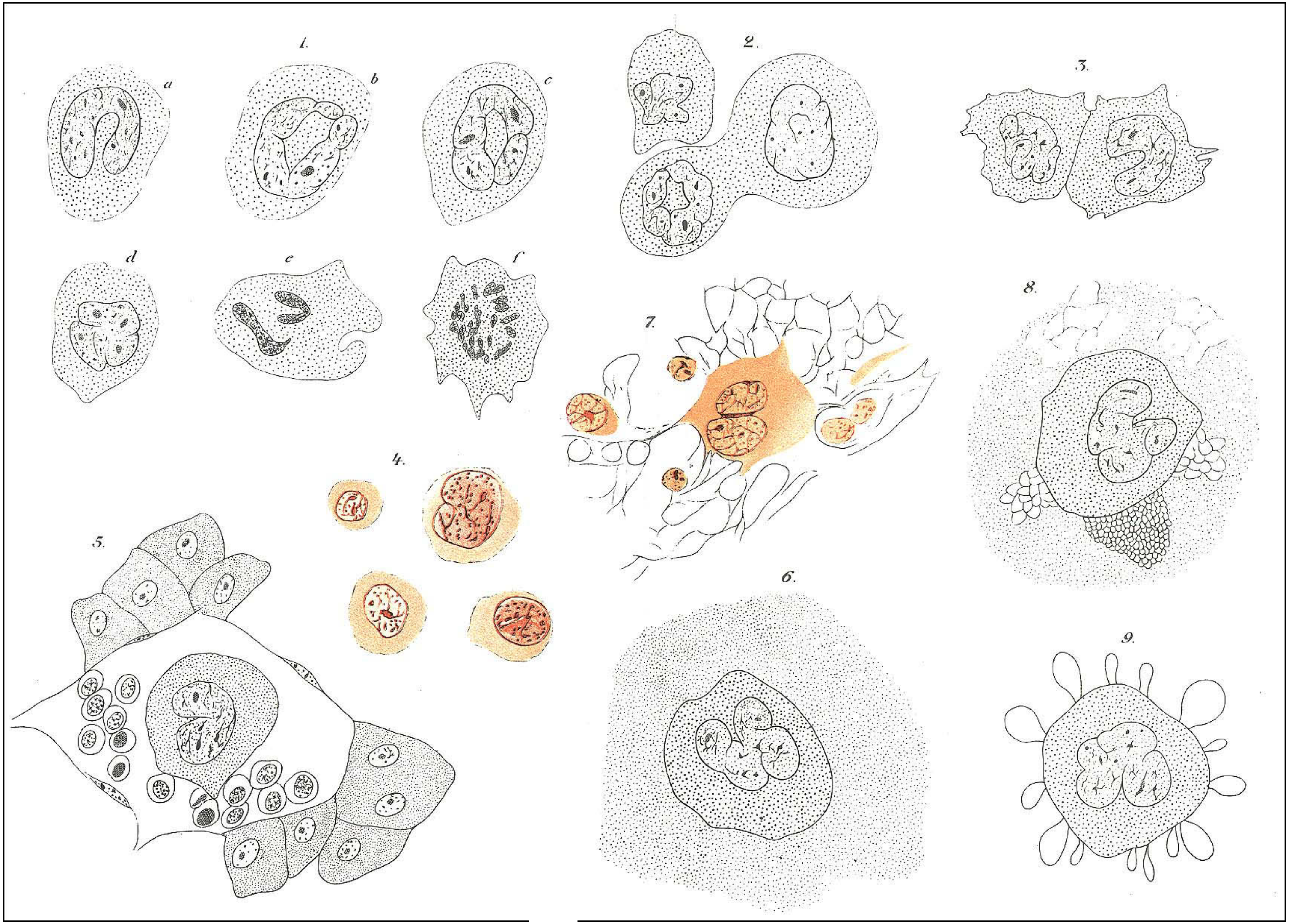
FIG. 6. Section of the hind leg of the same embryo, to show a megakaryocyte lying in a developing blood-vessel of the muscular tissue. On one side there is still a solid cord of erythroblasts, with some nucleated red corpuscles; on the other the blood plasma and fully formed nucleated red corpuscles lie in contact with the giant cell.

FIG. 7. Camera lucida sketch from the section of the femur of the fœtal cat 9 cms. to show the reticulum radiating from the megakaryocytes found in the marrow.

FIG. 8. A megakaryocyte surrounded by its broad envelope of secreted material from a preparation of the marrow of a young kitten, teased in a weak solution of methyl green in normal salt.

FIG. 9. The same cell, showing the vacuolation that takes place in the enveloping substance. The vacuoles at first small, as on the under side of the cell, become larger, until they form a structure resembling somewhat the reticulum shown in Fig. 7. The sketches of the vacuolation were made at different times, and have been shown in the same figure to indicate the gradual growth.

FIG. 10. A megakaryocyte from a similar preparation, in which the action of the reagent had gone so far before the cell was examined, that a number of vesicles adhering to the cell was all that remained of the original envelope.



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